

1262731

# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE  
United States Patent and Trademark Office

*December 16, 2004*

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 60/465,216  
FILING DATE: April 25, 2003  
RELATED PCT APPLICATION NUMBER: PCT/US04/12730

Certified by



Jon W Dudas

Acting Under Secretary of Commerce  
for Intellectual Property  
and Acting Director of the U.S.  
Patent and Trademark Office



BEST AVAILABLE COPY

Deposit Account # not found

1C006 U.S. PTO

PTO/SB/18 (10-01)

Approved for use through 10/31/2002. OMB 0651-0032  
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

### PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No.

1C996 U.S. PTO  
60/465216

04/25/03

INVENTOR(S)					
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)			
Quinn Q.	Tang	Gaithersburg, MD			
Patrick Y.	Lu	Rockville, MD			
Frank Y.	Xie	Germantown, MD			
Martin	Woodle	Bethesda, MD			

☐ Additional inventors are being named on the \_\_\_\_\_ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max):

RNAi Agents for Anti-SARS Coronavirus Therapy

PATENT TRADEMARK OFFICE

Direct all correspondence to:

☒ Customer Number 26633 → 26633

OR Type Customer Number here

☐ Firm or Individual Name

Address

Address

City

State

ZIP

Country

Telephone

Fax

ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages 10 ☐ CD(s), Number

☒ Drawing(s) Number of Sheets 4 ☐ Other (specify)

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 26633

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT (\$): 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: \_\_\_\_\_

Respectfully submitted,  
SIGNATURE

Date 4/25/03

TYPED or PRINTED NAME Paul M. Booth

REGISTRATION NO.  
(if appropriate)

40,244

TELEPHONE 202-912-2000

Docket Number:

38147-0022

Quinn Q. Tang  
Patrick Y. Lu  
Frank Y. Xie  
Yijia Liu  
Martin Woodle

## **RNAi Agents for Anti- SARS Coronavirus Therapy**

### **Background:**

A new disease called severe acute respiratory syndrome (SARS) has recently been reported in Asia, North America, and Europe. As of April 13, 2003 about 190 cases of SARS had been reported in the United States. In general, SARS begins with a fever greater than 100.4°F (>38.0°C). Other symptoms may include headache, an overall feeling of discomfort, and body aches. Some people also experience mild respiratory symptoms. After 2 to 7 days, SARS patients may develop a dry cough and have trouble breathing. The primary way that SARS appears to spread is by close person-to-person contact.

Most cases of SARS have involved people who cared for or lived with someone with SARS, or had direct contact with infectious material (for example, respiratory secretions) from a person who has SARS. Potential ways in which SARS can be spread include touching the skin of other people or objects that are contaminated with infectious droplets and then touching your eye(s), nose, or mouth. This can happen when someone who is sick with SARS coughs or sneezes droplets onto themselves, other people, or nearby surfaces. It also is possible that SARS can be spread more broadly through the air or by other ways that are currently not known.

Because the etiology of these illnesses has not yet been determined, no specific treatment recommendations can be made at this time. Empiric therapy should include coverage for organisms associated with any community-acquired pneumonia of unclear etiology, including agents with activity against both typical and atypical respiratory pathogens. Treatment choices may be influenced by severity of the illness. Infectious disease consultation is recommended.

Scientists in many laboratories in Asia, Europe and North America have been working on the cause of SARS around the clock. A previously unrecognized coronavirus in patients with SARS has been isolated, sequenced and tested in a monkey model. This new coronavirus, which is the leading candidate for causing SARS, has been named SARS coronavirus by the World

Health Organization. However, other viruses are still under investigation as potential causes of SARS.

Currently, five genome sequences of the SARS coronavirus have been reported by research groups in Canada, Hong Kong, and elsewhere. The sequences provide critical information for designing primers for RT-PCR assay as diagnosis tool. More importantly, based on these sequences, we designed small interfering RNA (siRNA) duplexes to knock down several viral proteins in order to inhibit the replication process of SARS coronavirus. This success in generating such siRNA duplexes permits development of siRNA based therapeutics for delivery into patient airways for both prevention and therapeutic treatment of SARS coronavirus infection.

#### **Rationale:**

SARS coronavirus is a segmented RNA virus, containing single stranded sense RNA, and causes one of the most prevalent infections in humans. The virulence of the SARS coronavirus results from i) its easy spread by aerosol and other person-to-person contacts, ii) its ability to escape from protective immunity by frequent changes in viral antigens (antigenic drift, like influenza virus), and iii) the sharp emergence of new virulent strains of the virus by, maybe, reassortment or mixing of RNA segments between viruses from two different species (antigenic shift). The threat of this new strain of SARS coronavirus is so severe because, despite intensive efforts, no effective therapy or vaccine is yet available for prevention and treatment of the SARS coronavirus infection.

RNA interference (RNAi) is a process by which double-stranded RNA directs sequence-specific degradation of messenger RNA in animal and plant cells. Studies have shown that in mammalian cells and animal models, RNAi can be triggered by synthetic 21-nucleotide duplexes of short interfering RNA (siRNA) and other types of double-stranded RNA molecules, such as, miRNA, shRNA, dsRNA, produced by expression vectors or *in vitro* transcriptions. There is a line of evidence that siRNA can effectively knockdown viral proteins of a group of RNA viruses, HIC, HCV and influenza, etc., resulting in significant effects of anti-viral infection in various mammalian cell systems and animal model systems.

We designed siRNA duplexes that potently inhibit SARS coronavirus production in cultured cells and animal models. To use these RNAi duplexes for prophylaxis and therapy of

SARS coronavirus infection in humans, the siRNAs must be delivered into epithelial cells in the upper airway and the lung, where the virus infection normally occurs. We have extensive experience in nucleic acid delivery in the pulmonary system for therapeutic development, which has previously been described. See PCT/EP00/13297, and US Patent Application Serial No.: 09/473,018, the contents of which are hereby incorporated by reference in their entirety. The methods described in PCT/EP00/13297 may be used to deliver the RNAi compositions described herein for therapeutic applications against SARS.

#### **Field of the Invention:**

The present invention provides compositions and methods that are useful for the treatment of severe acute respiratory syndrome (SARS). More specifically, RNAi agents that target SARS coronavirus are described, together with their use in methods clinical therapy of SARS and for bio-defense.

#### **Summary of Invention:**

The present invention provides novel RNAi agents having anti-SARS-coronavirus activity that knock down viral production of the key proteins required for SARS coronavirus replication and infection, and that disrupt the viral genome RNA directly. The invention provides: (1) sequences of RNAi agents, including chemically synthesized siRNA duplexes, in vitro transcribed dsRNA and vector expressed shRNA, siRNA, miRNA and dsRNA molecules, having anti-SARS coronavirus activity in mammalian cells, animal models and humans, that are useful for therapy; (2) examples of the target sequences coding for key proteins required for the virus replication and infection; (3) examples of the target sequences for RNAi-mediated disruption of the viral RNA genome in the non-coding regions; (4) routes of delivery for RNAi agents for animal models and humans; (6) examples of siRNA-mediated specific gene knockdown in mouse airways; (7) reagents that enhance the delivery and effectiveness of RNAi for anti-SARS coronavirus activities; and (8) methods and reagents for RNA template-specific RNA based RT-PCR for detection of any portion of the viral RNA genome, for applications of diagnosis and prognosis.

## **Detailed Description of the Invention:**

### **I. Key proteins required for replications and infections:**

Since little is known about the new SARS coronavirus gene functions and genomic components at present time, genome structure information from a previously defined virus, Dengue fever virus (DEN), was used to identify open-reading frames for key proteins of the newly identified coronavirus genome sequences.

The DEN virus was chosen because DEN virus is similar to coronavirus in that they both are positive single-strand RNA virus, and it has been reported that DEN virus replication was inhibited by siRNA targeting of the prM gene of DEN virus. Based on the previously known information about the genome structure of DEN virus and published SARS coronavirus genome sequences, three putative open reading frames of key proteins were identified as targets for siRNA-mediated knockdown: nsp1, a processing enzyme for protein maturation; nsp9, an RNA dependent RNA polymerase and important for RNA genome replication and for production of sub-genomic mRNAs; and S protein (spike), a surface glycoprotein for receptor binding, cell fusion, induction of neutralizing antibody and cellular immunity.

### **II. Design of siRNA duplexes**

Template viral genome sequences: SARS-CUHM-WI (AY278554, GI:30023518) was used for selection of the specific siRNA duplexes targeting to the corresponding genes (open reading frames). The targeted genes are listed as following:

Targeted genes:

nsp1: Coding for proteinase,

nsp9: Coding for RNA-dependent RNA Polymerase (RdRp), the sequence of SARS-CUHM-WI and SARS-Tor2 are identical.

S: Coding for spike protein that binds to cell receptor, induce fusion, and induce neutralizing Ab and T-cell immunity. There are 3 bp non-homologous to SARS-To2, which were avoided when designing siRNA duplexes.

Two siRNA duplexes were designated for each targeted genes based on Tuschl's guidelines (see M. T. McManus and P. A. Sharp, Gene silencing in mammals by small interfering RNAs. *Nature Reviews, Genetics*. 3, 737 (2002), the contents of which are herein incorporated by reference in their entirety). The sequences and locations of these siRNA oligos

are listed in the Table 1 and Table 2. Figure 2 shows a map of the SARS coronavirus genome structure with the positions of the targeted sequences.

**Table 1. Sequences of siRNA targeting coronavirus**

All target sequences underwent a BLAST search for potential cross-talk to non-related sequences. The sequences shown below are all unique sequences that are homologous only to the published SARS coronavirus sequences including strains of SARS-Urbani and SARS-Tor2.

<u>Genes</u>		<u>Targeted sequences (5'-3')</u>	<u>Locations</u>
nsp1	1	AACCTTTGGAGAAGATACTGT	2711-2731 nt
	2	AATCACATTTGAGCTTGATGA	2762-2782 nt
nsp9	1	AAGTTGCTGGTTTTGCAAAGT	13467-13487 nt
	2	AAGGATGAGGAAGGCAATTTA	13520-13540 nt
S (spike)	1	AAGCTCCTAATTACACTCAAC	21543-21563 nt
	2	AATGTTACAGGGTTTCATACT	21659-21679 nt

**Table 2. Locations of Selected Targets in Virus Genomes**

Two siRNA duplexes were selected to target each of the putative open reading frames.

<u>SARS-CUHK</u>		<u>Position on SARS-Urbani</u>	<u>Gene of SARS-Urbani</u>
nsp1	1	2736-2756 nt	nsp-popypprotein pp1a/pp1ab
	2	2787-2807 nt	
nsp9	1	13492-13512 nt	nsp-popypprotein pp1a/pp1ab
	2	13545-13575 nt	
S(spike)	1	21568-21588 nt	Spike Protein
	2	21684-21704 nt	

Additional SARS coronavirus sequences are being described. The targeted sequences we selected have 100% homology to the most of those strains in the corresponding regions, except HKU39849 (Figure 3).

In addition to the above examples, other open reading frames and non-coding regions in the SARS coronavirus can also be targeted by specific RNAi agents for effective eradication of the coronavirus infection and replication.

### III. RS-PCR for detection of SARS coronavirus

A unique RT-PCR assay called RNA template specific PCR (RS-PCR) has been designed for detection of SARS coronavirus RNA.

- a. An RS-PCR based SARS diagnosis assay uses primers for detecting the SARS coronavirus sequences. Briefly, the assay uses a SARS coronavirus gene specific primer (SRT primer) which contains a 17 nt sequence complementary to the SARS coronavirus sequence and a special sequence of 30 nt attached to its 5' for the reverse transcriptase (RT) synthesis of the first strand of cDNA from RNA of the SARS coronavirus genome. A pair of primers was then used for PCR amplification. The forward primer (Forw-primer) recognizes a sequence in the SARS coronavirus genome upstream of the 17 nt region recognize by the SRT primer. The reverse primer (Rev-primer) recognizes the special sequence attached to the SRT primer. The PCR amplification was performed at high annealing temperature (72°C) at which only the cDNA from RT can be amplified but not any potential DNA contamination. The RS-PCR assay can be easily scaled up for large-scale application on diagnosis and prognosis.

- b. RS-PCR Primers Design:

Primer 1: Forward-nsp1Up (30-mer, 41-70 nt of the putative nsp1 gene coding sequence, or 2734-2763 nt of coronavirus sequence, AY278554 ,)

5'--- GGG AAG TTC AAG GTT ACA AGA ATG TGA GAA---3'

Primer 2: SRT-nsp1Dn (47-mer, the 17-mer at 3' is complementary to 1041-1025 nt of the putative nsp1 gene coding sequence, or 3734-3718 nt of coronavirus sequence, AY278554)

5'--- GAA CAT CGA TGA CAA GCT TAG GTA TCG ATA gac aac ctg ctc ata aa---3'

Primer3: Forward-nsp9Up (30-mer, 35-64 nt of the putative nsp9 gene coding sequence, or 13381-13410 nt of coronavirus sequence, AY278554)

5'---CGG TGT AAG TGC AGC CCG TCT TAC ACC GTG---3'



Primer4: SRT-nsp9Dn (47-mer, the 17-mer at 3' is complementary to 734-718 nt of the putative nsp9 gene coding sequence, or 14080-14064 nt of coronavirus sequence, AY278554)

5'--- GAA CAT CGA TGA CAA GCT TAG GTA TCG ATA gag gat ggg cat cag  
ca---3'

Primer5: Forward-SpikeUp (30-mer, 45-74 nt of coding sequence of the putative Spike gene coding sequence, or 21511-21540 nt of coronavirus sequence, AY278554)

5'---CCT TGA CCG GTG CAC CAC TTT TGA TGA TGT---3'

Primer6: SRT-SpikeDn (47-mer, the 17-mer at 3' is complementary to 644-628 nt of the putative Spike gene coding sequence, or 22110-22094 nt of coronavirus sequence, AY278554)

5'--- GAA CAT CGA TGA CAA GCT TAG GTA TCG ATA gtg tta aaa cca gaa  
gg---3'

Primer 7: (Rev-primer)

5'-AACATCGATGACAAGCTTAGGTATCGATA-3'

#### c. RS-PCR

The following procedure is used for RS-PCR to detect SARS coronavirus in biological samples such as cell lysates, animal tissue and human patient tissue. Other tissues may also be used.

1) Total RNA is isolated from human sample using RNAwiz™ reagent (Ambion). MuLv Reverse Transcriptase and RNase inhibitor are available from Applied Biosystems and all other reagents used in the RS-PCR are available from PE Biosystems.

2) SRT reaction: 1 µg of total RNA sample is mixed with 2 µ of 10X PCRII buffer, 4 µl of 25 mM MgSO<sub>4</sub>, 0.5 µl of 10 mM dNTPs, 1 µl RNase inhibitor (20 U/µl), 1 µl of 20 uM SRT primer, 1 µl of MuLv reverse transcriptase (50 U/ul), and RNase free water to a total

volume of 20  $\mu$ l. The sample is incubated at 37°C for 30 minutes followed by at 42°C for 15 minutes, then heated at 94°C for 5 minutes.

3) PCR: 10  $\mu$ l of SRT product is mixed with 4  $\mu$ l of 10X PCRII buffer, 3  $\mu$ l of 25 mM MgSO<sub>4</sub>, 1  $\mu$ l of 10mM dNTPs, 1  $\mu$ l of 20 uM Forw-primer, 1  $\mu$ l of 20 uM Rev- primer, 0.5  $\mu$ l of Taq DNA polymerase (5 U/ $\mu$ l), and distilled water to a total volume of 50  $\mu$ l. The sample is heated at 94°C for 2 minutes, and then subjected to 35 cycles of 2- step PCR: 94°C for 1 minutes, annealing and extension at 72°C for 2 minutes. An extra 10 minutes incubation at 72°C is allowed at the end of PCR followed by incubation at 4°C, before the PCR products are analyzed by running 10  $\mu$ l RS-PCR product in a 0.8% agarose gel.

**Table 3. Lengths of RS-PCR Products**

	Gene	Primer For SRT	Primers For PCR	Size of RS- PCR products
1	nsp1	2	1+7	1031 bp
2	nsp9	4	3+7	730 bp
3	S protein	6	5+7	630 bp

#### **IV. Pulmonary siRNA Delivery**

There are multiple routes for effective nucleic acid delivery into mammalian airways (see Figure 4). We have developed an oral-tracheal delivery for siRNA duplex and other nucleic acid for effective gene expression manipulations (Figure 5). The unique formulations related to this type of delivery include surfactant, liposome and peptide polymers. The nasal delivery and other types of airway delivery methods are also applied for achieving the most effective nucleic acid delivery. When fluorescein-labeled siRNA duplexes were administrated into the upper airway through nasal delivery and lower airway through oral-tracheal delivery, the duplexes were observed in both trachea and lung, even after intensive washing (Figure 6 and 7).

What is claimed is:

1. An isolated double stranded RNA molecule comprising a first strand comprising a ribonucleotide sequence which corresponds to a nucleotide sequence of a SARS virus and a second strand comprising a ribonucleotide sequence which is complementary to the nucleotide sequence of said SARS virus gene, wherein said double-stranded molecule inhibits expression of said nucleotide sequence of said SARS virus.
2. The RNA molecule according to claim 1 wherein said first and second strands are separate complementary strands.
3. The RNA molecule according to claim 1 wherein said first and second strands are contained in a single molecule, wherein said single molecule comprises a loop structure.
4. The RNA molecule according to any preceding claim wherein said nucleotide sequence of a SARS virus is selected from the group consisting of an nsp1 sequence, an nsp9 sequence and a spike sequence.
5. The RNA molecule according to claim 4, wherein said first strand comprises a sequence selected from the group consisting of AACCTTTGGAGAAGATACTGT, AATCACATTTGAGCTTGATGA, AAGTTGCTGGTTTTGCAAAGT, AAGGATGAGGAAGGCAATTTA, AAGCTCCTAATTACACTCAAC, and AATGTTACAGGGTTTCATACT.
6. A method of detecting a SARS virus in a sample, comprising (a) contacting RNA obtained from said sample with a gene specific primer comprising a 3' region that is complementary to a SARS sequence and a 5' sequence that is not complementary to a SARS sequence and synthesizing a first strand cDNA molecule by reverse transcription followed by (b) amplifying said first strand cDNA in a PCR using a pair of primers, wherein the first primer is complementary to said 5' region of said gene specific primer and wherein the second primer comprises a sequence in the SARS genome that is upstream of the region recognized by said 3' region of said gene specific primer, and (c) detecting the product of said PCR.

7. The method of claim 6 wherein said gene specific primer is complementary to a SARS nps1, nps9 or spike sequence.

8. The method of claim 7, wherein said gene specific primer comprises a sequence is selected from the group consisting of

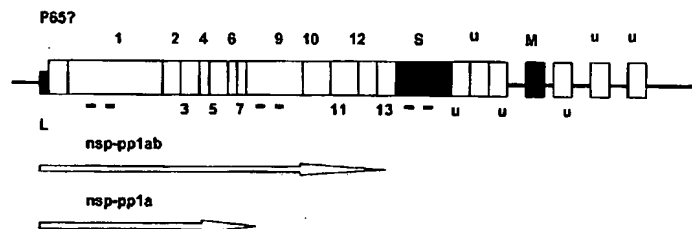
GAA CAT CGA TGA CAA GCT TAG GTA TCG ATA gac aac ctg ctc ata aa,  
GAA CAT CGA TGA CAA GCT TAG GTA TCG ATA gag gat ggg cat cag ca, and  
GAA CAT CGA TGA CAA GCT TAG GTA TCG ATA gtg tta aaa cca gaa gg.

9. The method of claim 8, wherein said first primer comprises the sequence  
GAACATCGATGACAAGCTTAGGTATCGATA

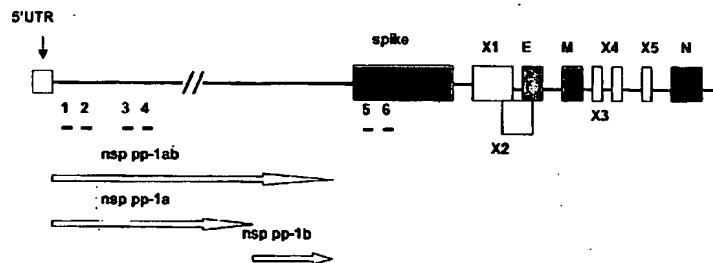
10. The method of claim 6 wherein said second primer comprises a sequence selected from the group consisting of

GGG AAG TTC AAG GTT ACA AGA ATG TGA GAA,  
CGG TGT AAG TGC AGC CCG TCT TAC ACC GTG, and  
CCT TGA CCG GTG CAC CAC TTT TGA TGA TGT.

V. Figures



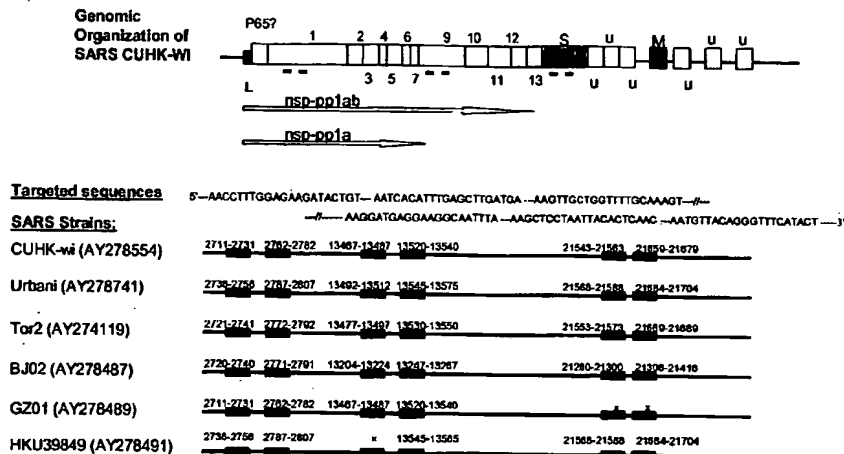
**Fig 1. Genomic Organization of SARS Coronavirus CUHK-WUrbani** Genomic sequence of the SARS coronavirus CUHK-WUrbani strain (AY278554.1), which is 29206 bps long. The sizes of the genes are drawn about to scale. Structural proteins are shown as solid box. L, leader sequence; "p65?" indicates putative MHVp65-like protein; number 1-13 show non-structural (nsp) proteins, where nsp-8 is missing in published sequence data. S, spike protein; M, membrane glycoprotein; U, unknown proteins. Arrows show non-structural polyproteins. Black bars show the position of the siRNA-targeted sequences.



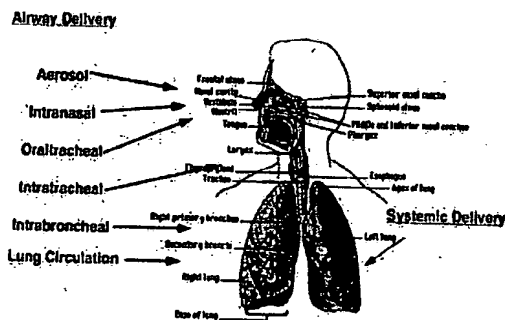
**Fig 2. Genomic Organization of SARS Coronavirus Urbani**

Genomic sequence of the SARS coronavirus Urbani strain (AY278741.1), which is 29727 bps long. The sizes of the genes are drawn about to scale. Structural proteins are shown as solid box. S, spike proteins; E, envelope protein; M, membrane glycoprotein; N, nucleocapsid phosphoprotein. Arrows show non-structural polyproteins. Numbered black bars show the position of the siRNA-targeted sequences.

Title: RNA<sub>i</sub> Agents for Anti- SARS  
 Coronavirus Therapy  
 Serial No: Unassigned  
 First Inventor: Quinn Q. Tang  
 Docket No: 38147-0022



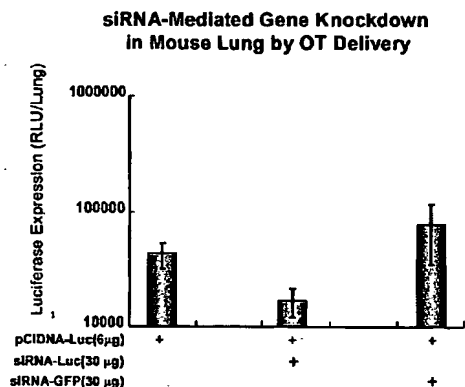
**Fig. 3 Location of siRNA targets on different SARS coronavirus isolates** Target sequences as designed based upon SARS coronavirus CUHK-WI were used to find its specificity for different SARS coronavirus isolates. The "mis-match" of the fifth and sixth target sequences (Spike-1 & 2) with GZ-01 isolate was simply because the incomplete sequence data of GZ01 isolate as submitted; and the mis-match of the third target sequence (nsp9-A) on HKU39849 was because there is one base pair missing in HKU39849 sequence at position 13486 nt, which was not found in genomic sequence of other isolates.



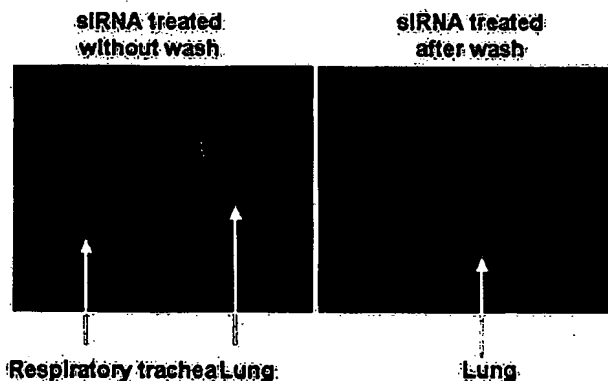
**Figure 4. Nucleic acid delivery to pulmonary system.** Airway delivery is very effective through multiple routes. Aerosol, intranasal installation and oral-tracheal delivery are the non-invasive approaches for pre-clinical study with animal models, and later for human clinical studies.

Title: RNAi Agents for Anti- SARS  
 Coronavirus Therapy  
 Serial No: Unassigned  
 First Inventor: Quinn Q. Tang  
 Docket No: 38147-0022

60465216 .042503



**Figure 5. Inhibition of luciferase expression by siRNA in the lung.** Luciferase plasmid together with siRNA specific for either GFP or luciferase were orotracheally into mice, using either 5% glucose or Infasurf. Luciferase activity was measured 16 hrs later in lung homogenates.



**Figure 6. Distribution of fluorescence-labeled siRNA in the respiratory tract of mice using Nostril delivery route** Thirty ug of fluorescein-labeled siRNA duplex in 50 ul Nostril delivery solution ( 5% glucose and 12 ug/ul infasurf) was delivered into the respiratory tract through the Nostril delivery route. Four hours post delivery, the animal was sacrificed and the respiratory trachea and lung were isolated. Examination of tissues under fluorescence microscopy revealed massive distribution of siRNA in the respiratory tract and lung, even after washing tissues with PBS to remove siRNA non-specifically attached to cell surface.

Title: RNAi Agents for Anti-SARS  
Coronavirus Therapy  
Serial No: Unassigned  
First Inventor: Quinn Q. Tang  
Docket No: 38147-0022

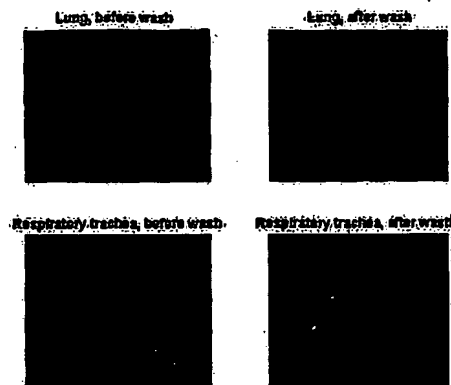


Figure 7. Distribution of fluorescence-labeled siRNA in the respiratory tract of mice using Oral-tracheal delivery route. Thirty ug of fluorescein-labeled siRNA duplex in 50 ul Oral-tracheal delivery solution (5% glucose and 12 ug/ul intasurf) was delivered into the respiratory tract through the Nasal delivery route. Four hours post delivery, the animal was sacrificed and the respiratory trachea and lung were isolated. Examination of tissues under fluorescence microscopy revealed massive distribution of siRNA in the respiratory trachea and lung, even after washing tissues with PBS to remove siRNA non-specifically attached to cell surface.



# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/012730

International filing date: 26 April 2004 (26.04.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US  
Number: 60/465,216  
Filing date: 25 April 2003 (25.04.2003)

Date of receipt at the International Bureau: 23 December 2004 (23.12.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

This Page is inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☒ BLURED OR ILLEGIBLE TEXT OR DRAWING
- ☒ SKEWED/SLANTED IMAGES
- ☐ COLORED OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☒ REPERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images  
problems checked, please do not report the  
problems to the IFW Image Problem Mailbox**